REMARKS/ARGUMENTS

In response to the Final Office Action of March 31, 2008 in which the Examiner rejected claims 13 and 14 under 35 U.S.C. § 102(b) and double patenting. Claim 13 has been amended herein. Claims 38-49 are newly added. Specific support for these amendments is found in the as-filed specification. Specifically, the generation of directed genetic modification is provided in the specification at, for example, [0039], [0096]-[0097] and [0112] for example. The development of compensatory metabolic pathways is discussed in the specification at, for example, [0250], [0302], [0364], [0369]-[0373] and [0530] as well as being specifically the topic of Examples F.1 and F.1.2. The development of such compensatory metabolic pathways is further specifically illustrated in Figures 2 and 4. The isolation and characterization of the evolved protein is described in [0479] et seq. Thus, no new matter is added by way of these amendments.

Examiner Interview Summary

On June 23, 2008 Attorneys Fairman and Franck Tetaz (FR) along with inventor Philippe Soucaille had an interview with Examiners Long, Woitach and Epps-Ford (attending by phone). Dr. Soucaille described the invention and attorneys Fairman and Tetaz described the drafting of the specification and further, that the instant specification was translated from the original French. The applicants discussed the claim language and the art cited in the previous office actions. No agreement was reached. However, the applicants wish to thank the Examiners for their time and very helpful comments.

On July 17, 2008 Attorney Fairman had a telephonic interview with Examiner Long. The participants discussed proposed claim amendments for further prosecution. No agreement was reached. Applicants wish to thank Examiner Long for his helpful comments.

Rejection under 35 U.S.C. § 102(b)

Claims 13 and 14 were rejected under 35 U.S.C. § 102(b) as being anticipated by Nakamori et al. (Applied Microbial Biotechnology, 1999; 52: 179-185).

This rejection is overcome, at least, for the following reasons.

<u>Nakamori Cannot Anticipate Because It Does Not Teach A Directed Genetic Modification Or The Inhibition Of The Production Or Consumption Of A Substrate</u>

The claims require generating a genetic modification in a gene of interest that inhibits the production or consumption of a metabolite by directed mutation. Nakamori, in contrast, uses random mutagenesis to generate thousands (at least) of random mutagenic events. Nakamori then eventually identifies some cells in which an overexpressor phenotype of methionine is identified. Further genetic analysis eventually finds that the repressor gene *metJ* is repressed. (Abstract, entire paper). As discussed by Nakamori, and the instant specification at for example, [0214], [0237], methionine and cysteine act in a negative feedback manner on the *metJ* gene to inhibit the production of methionine. When Nakamori mutates (randomly) the *metJ* gene, the result is the increased production of methionine by ***<u>autotrophic</u>*** superproducers on ***<u>complete media</u>*** increasing the production of methionine. See, pg. 181, col. 2, results and pg. 182, col. 1, especially, Table 2 and pages 183-185. There is no substrate or product that is inhibited. In Nakomori, there is merely a de-repression of the repressor gene. This is succinctly stated in the instant specification at, for example, [0214]. This is also stated in Japanese patent application JP 2000157267 to Nakamori (believed to encompass the research of the Nakamori reference cited by the Office) and cited in the applicants Information Disclosure Statement. Further, this is stated in the Nakamori application (JP 2000157267) as the problem to be solved. "To obtain a new variation type *metJ* gene having a specific amino acid sequence lowered in activity in *repressing* methionine biosynthesis." JP 200015726 (Nakamori, Abstract). Therefore, Nakamori cannot anticipate the instant invention at least because the instant claims ***require*** "generating a genetic modification in a gene of interest wherein the production or consumption of a substrate is inhibited." As discussed in Nakamori, no production or consumption of any substrate is inhibited and only one of the, at least, millions of random mutagenic events generated 5500 were identified as analogue resistant and of these just seven were halo forming using the *pediococcus acidilactici* methionine assay. Of these seven only four produced appreciable quantities of methionine. Nakamori Table 1. Further, this assay provides a complete medium, excepting methionine, the production of which, by the inoculum allows the *pediococcus acidilactici* to grow. See, Appendix I. Thus, the procedure of Nakamori is hardly directed genetic mutation and no cells (from the random mutagenic event) were selected in which the production or consumption of a substrate was inhibited. In fact, the opposite selection

criteria were used, cells were selected in which the production was de-repressed. For these reasons alone, the rejection is overcome and should be withdrawn. Applicants respectfully request the same.

Nakamori Teaches Against the Instant Invention

As discussed above, the instant invention teaches a method of producing an evolved microorganism requiring, in part, the step of generating a microorganism wherein the production or consumption of a substrate is inhibited. In contrast, Nakamori explicitly states: "The construction of mutants that have a genetically altered regulatory mechanism ***is probably essential*** for the fermentative production of L-methionine." Pg. 179, 2nd column, last sentence (emphasis added). This statement is made with regard to the repressor genes *metJ* and *metK*. Thus, the ***essential*** requirement, as taught by Nakamori, is the removal of the negative feedback exerted by the *metJ* gene. There is no evolution of a "compensatory metabolic pathway" as is required by the instant claims. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

The Process of Nakamori Does not Result in a Compensatory Metabolic Pathway

As discussed above and as required by the claims, the instant invention discloses the *directed* genetic modification of a gene of interest wherein the production or consumption of a metabolite is inhibited such that a compensatory metabolic pathway is evolved. In Nakamori, there is no difference in the pathway responsible for methionine synthesis other than the negative feedback mechanism is eliminated. No new substrates are available to the organism for methionine synthesis and no compensatory pathways are necessary (or indeed ever evolved). In Nakamori, the wild type pathway continues to produce methionine using pre-mutagenic synthesis steps. Thus, because the instant claims require the debilitation of a biosynthesis pathway and the evolution of a compensatory pathway, Nakamori cannot anticipate the instant invention. For this reason alone, the rejection is overcome and should be withdrawn.

Nakamori Teaches A Different Process Having Different Steps

As amended herein, the present invention claims a method for preparing an evolved protein comprising the steps of:

a) generating a directed genetic modification in a gene of interest in an initial microorganism to yield a modified microorganism wherein the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium, wherein the ability of the modified microorganism to grow is impaired;

b) culturing the modified microorganism obtained in step (a) on the defined medium, allowing the modified microorganism to evolve a compensatory metabolic pathway to compensate for the impaired growth, wherein the defined medium can contain a co-substrate promoting the evolution; and

c) selecting an evolved microorganism from step (b) able to grow on said defined medium;

wherein a compensatory metabolic pathway is evolved allowing the evolved microorganism to proliferate on the defined medium.

wherein at least one protein in the compensatory metabolic pathway has evolved allowing the modified microorganism to proliferate.

As described in the specification at, for example, [0011]-[0116], the genetic modification can at least include: (1) expressing a wild type gene that is otherwise suppressed; (2) expressing a heterologous gene that includes a sequence under the control of regulatory elements that permit its expression and translation in the original strain; (3) obtained by directed mutagenesis directly on the wild type gene present naturally in the initial strain, for example, by homologous recombination; or (4) obtained by other directed mutagenesis. Thus, those of skill in the art will appreciate that the ability to carry out any of the aforementioned procedures *requires* that the user identify and specifically determine which gene will be modified. For example, the process of homologous recombination cannot be performed without knowing the specific genetic sequence of the insertion point on the genome and the specific sequence of the genes being inserted. Further, such processes are taught in the specification at, for example, [0117]-[0120], as described in the specification. The "directed evolution" of the microorganism is then performed as described in the specification, at for example, [0231]-[0235] and in the Examples, provided from [0375] to [0421].

For example, these methods include a "coupling of the biosynthesis of the substance of interest with the growth of the micro-organism in such a way that the substance becomes necessary for the growth of the microorganism." [0232]. For example, for the production of methionine, the gene for methionine synthase is disrupted which results in the micro-organism

becoming auxotrophic for methionine. As explained above, to survive in a minimum medium containing a simple carbon source and methyl mercaptan or sodium methyl mercaptide, the micro-organism has therefore to optimize the synthesis of L-methionine from O-acyl-L-homoserine and methyl mercaptan or sodium methyl mercaptide. [0234].

As explained in the specification, the induced auxotrophy results in an "evolved pathway" resulting from the "modified" activities of, for example, 'modified' methionine synthase [0069]-[0253], 'modified' cysteine synthase [0259]-[0307] and 'modified' NADPH synthesis [0308]-[0340] e.g. a compensatory pathway is evolved whereby at least one enzyme with a "modified activity results remediates the induced auxotrophy. See, FIGURES and EXAMPLES [0341]-[0653]. In these examples, the native activity of the initial non-evolved enzyme was not a methionine synthase, cysteine synthase, or NADPH synthase, it was, for example cystathionine γ-synthase that evolved into "methionine synthase", cystationine γ synthase that evolved into homocysteine synthase, O-acetyl-homoserine synthase that evolved into homocysteine synthase or O-acetyl-homoserine synthase that evolved into methionine synthase, for example. See, Fig. 2 and [0342]-[0350]. Thus, as explained it is possible to generate a genetic modification in a gene of interest e.g., "it is possible to obtain, ***in a controlled manner***, a modification of the substrate specificity of the enzyme" [0278] (emphasis added). As described, "[T]he strains modified according to the invention are genetically modified by the inactivation, mutation and/or over activation of at least one endogenous gene in order to permit the evolution of a new metabolic pathway" (e.g., a compensatory metabolic pathway). [0280]. As explained "the strain modified in this way is preferably selected and improved by a method of screening and evolution" making it possible, for example "to cause the acyl-homoserine sulfhydrylase activity to evolve into a cysteine synthase activity to restore the production of cysteine." [0284]. "The transformation of the acyl-homoserine sulfhydrylase activity into an "evolved cysteine synthase activity is deemed to be achieved when the genetically modified and evolved bacterial strain (E) has a growth rate at least similar to that of the initial modified strain (M) when grown in a minimal medium in the presence of glucose as a single carbon source." [0285]. Thus, in some exemplary embodiments, "the transformation of the acyl-homoserine sulfhydrylase activity into the "evolved cysteine synthase" activity is deemed to be achieved when the cysteine synthase activity carried by the modified O-acyl-L-homoserine sulfhydrylase protein has been improved by 10% relative to its initial activity. [0285]. Further methods of

modification are provided in the specification at, for example, [0286]-[0292]. This is further succinctly stated in the Examples. "b) Culture of the above modified strain on the same minimal medium (MM) to which sodium methylmercaptide (co-substrate) has been added to cause the ***evolution*** of an endogenous enzyme activity into a methionine-synthase activity to ***compensate*** for the initially deleted enzyme activity (*metE*)." [0365] (emphasis added).

It should be emphasized that after the genetic modification is generated, the modified microorganisms are grown in a non-minimal media to provide a volume of *the* mutant bacteria. Not, as in Nakamori, where innumerable random mutations are made and a desired mutant is then selected for. Thus, as explained at, for example, [0390]: "b) Culture and evolution of the Δ(*metE*) Modified Strain in the Presence of Sodium Methylmercaptide as Co-Substrate. To optimize E. coli for the production of methionine from methylmercaptan, a controlled selection is carried out in flasks. . . . The controlled selection is conducted in a hermetically sealed glass flask containing 50 ml of inorganic medium in the presence of 33mM glucose and chloramphenicol at a final concentration of 25mg/l. The culture media are seeded with the strain *E. coli* K12 Δ*metE* at a defined value of OD_{600}nm. Seeding is carried out with a sufficiently large population of bacteria so that some bacteria potentially possess relevant spontaneous mutations in the gene *metB* enabling assimilation of methylmercaptan (e.g., compensating for the auxotrophy as explained at, for example, [0250], [0302], [0363], [370], [0374], [0530]. This population is obtained by culture of the strain auxotrophic for methionine on a minimal medium supplemented with methionine." "[T]hree flasks then receive 100 μl of a 400 mg/l solution of sodium mercaptide, while a fourth flask received no added sodium mercaptide." [0396]. As discussed in the specification, the process of culturing and selecting the evolved microorganisms requires culturing the bacteria for a period of time long enough to allow the bacteria to develop the compensatory metabolic pathways, e.g., for 4-6 days or more. [0396]. As illustrated by TABLE 2, it was not until 6 days of growth that the modified microorganisms evolved enough to produce the desired end product. Further, applicants note that for wild type bacteria, a single overnight culture would yield an OD of over 1.0. Thus, the ability to produce the evolved bacteria requires the gentle culture of the bacteria over many "generations" of bacterial growth. As defined, <u>Generate: to cause to exist; produce</u> (Cambridge Dictionary of American English, © Cambridge University Press 2008.) Thus, there can be no doubt that producing a modified

microorganism by directed genetic modification *a priori* requires generating a genetic modification in a gene of interest.

The instant method can then be summarized as follows:

1 – Directed genetic modification of a microorganism;

2 – Culturing only those micro organisms in which the directed genetic modification was made. Specifically, on a minimal media devised for the selection and evolutionary pressure of the desired biosynthetic pathway;

3 – Obtaining those microorganisms that grow in step 2.

Schematically, this procedure can be represented as:

$$\mathbf{I_0} \rightarrow \mathbf{M_0} \rightarrow \mathbf{E_0}$$

Where:

$\mathbf{I_0}$ is the initial microorganism;

$\mathbf{M_0}$ is the modified microorganism; and

$\mathbf{E_0}$ is the evolved microorganism.

In contrast, Nakamori does not teach "generating a directed genetic modification in a gene of interest" as required by the claims but rather, Nakamori teaches wild type E. coli that are mutagenized by N-methyl-N'nitro-N-nitrosoguanidine, *which induces random mutations*. Next, the mutagenized cells are spread onto plates containing various quantities of L-methionine analogs and incubated for 72 h. This step is performed to select cells that have acquired a mutation in the methionine biosynthesis pathway; however, this is not an evolutionary step. I.e., what Nakamori has done is merely randomly mutagenizing bacteria and selecting those that have become resistant to the L-methionine analogue ethionine. These resistant bacteria are then further selected for L-methionine overproduction.

The difference in Nakamori and the instant invention is succinctly illustrated in Fig. 1 of Nakamori as compared to Figs. 2 and 4 of the instant invention. As shown in Fig. 1 of Nakamori the native methionine biosynthetic pathway is intact merely the feedback inhibition has been eliminated. In contrast, as shown in Figs. 2 and 4 of the instant invention, induction of compensatory metabolic pathways (and enzyme) requires the disabling of the native gene (and

enzyme). Thus, as summarized in Fig. 2, the native methionine biosynthetic pathway is the central pathway (corresponding to Nakamori, Fig. 1). Further, as shown in the figure and discussed in the specification, the step at which the compensatory pathway is desired corresponds to the genetic mutation generated. Thus, when the *metC* gene is disabled the biosynthesis compensates with the evolution of either *metB*** or *metY**. When *metE* is disabled the biosynthesis compensates with an evolution of *metY*** or *metB**. Fig. 2, [0342]-[0350].

Thus, Nakamori can be summarized as follows:

1 – Random mutagenesis of bacteria

2 – Isolation of autotrophic superproducers on complete medium.

Schematically, this process can be represented as:

$$\mathbf{I_o} \rightarrow \mathbf{R_o}.$$

Where:

$\mathbf{I_o}$ is the initial microorganism;

$\mathbf{R_o}$ is the randomly mutagenized microorganism.

Therefore, as discussed above, Nakamori cannot anticipate the instant invention, at least because the instant invention requires that the initial organism be modified by (1) a directed genetic modification such that (2) the production or consumption of a substrate is inhibited. Nakamori teaches neither of these elements. For at least these reasons, the rejection of claims 13-14 over Nakamori is overcome and should be withdrawn. Applicants respectfully request same.

The Office Is Using Impermissible Hindsight To Identify The Instant Invention

The instant invention claims a method for producing an evolved protein comprising, in part, "generating a directed genetic modification in a gene of interest to yield a modified microorganism in which the production or consumption of a substrate is inhibited." Further, the claims requires the modified microorganism to be cultured on a defined medium so as to evolve a compensatory metabolic pathway to compensate for the impaired growth." Further, the claims require that "the evolved microorganism be cultured on a defined medium" Nakamori does none

of these things. Nakamori uses random mutagensis of millions of microorganisms to eventually select seven that have a the potentially desirable phenotype of which only 4 can be shown to actually produce methionine. Nakamori's mutants are not cultured on a defined medium but rather on a complete medium and consequently, there is no pressure to evolve. thus, lacking the elements of the present invention, applicants can only surmise that the Office is using hindsight reconstruction to find the elements of the instant claim in Nakamori. Courts have repeatedly warned that the patentability of an invention is not to be viewed with hindsight or "viewed after the event." See Goodyear Co. v. Ray-O-Vac Co., 321 U.S. 275, 279, 64 S.Ct. 593, 88 L.Ed. 721 (1944) and authorities cited therein. The Office is further reminded of the warning recently provided by the Supreme Court and as further cited by the Board of Patent Appeals. "[A] factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of argument reliant upon ex post reasoning." KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727 at 1742.

Double Patenting Rejection

Claims 13 and 14 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claim 1 and 12-13 of copending application number 10/781,499.

Applicants, respectfully request that this rejection be held in abeyance until allowable claims are identified in the present application and co-pending application 10/781,499 at which time applicants will file a terminal.

CONCLUSION

This application now stands in allowable form and reconsideration and allowance is respectfully requested.

This response is being submitted on or before July 30, 2008 with a $60.00 fee for a one-month extension of time making it a timely response. It is believed that no additional fees are due in connection with this filing. However, the Commissioner is authorized to charge any additional fees, including extension fees or other relief which may be required, or credit any overpayment and notify us of same, to Deposit Account No. 04-1420.

Respectfully submitted,

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